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Ambient Water Quality Criteria: Nitrosamines

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Section 304(a) of the Clean Water Act (33 U.S.C. 1314(a)), requires EPA to publish and periodically update water quality criteria. These criteria are to reflect the latest scientific knowledge on the identifiable effects of pollutants on public health and welfare, aquatic life, and recreation. This report presents water quality criteria for nitrosamines and nitrosamides including N-nitrosodiphenylamine, N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosodibutylamine, and N-nitrosopyrrolidine. It presents concentration criteria for the protection of fresh water and saltwater aquatic life. It presents 'safe' concentrations for humans, and in the case of suspect or proven carcinogens, gives various levels of incremental cancer risk. A section 304(a) water quality criterion is a qualitative or quantitative estimate of the concentration of a water constituent or pollutant in ambient waters which, when not exceeded, will ensure a water quality sufficient to protect a specified water use. Under the Act a criterion is a scientific entity, based solely on data and sci , ...

KEYWORDS: *Water quality, *Toxicology, *Nitroso compounds, *Water pollution effects(Humans), *Water pollution effects(Animals), *Toxic substances, *Nitrosoamines, *Diphenylamine/N-nitroso, *Dimethylamine/N-nitroso, *Diethylamine/N-nitroso, *Dibutylamine/N-nitroso.

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NITROSAMINES

Ambient Water Quality Criteria

Criteria and Standards Division
Office of Water Planning and Standards
U.S. Environmental Protection Agency
Washington, D.C.

CRITERION DOCUMENT

NITROSAMINES

Criteria

Aquatic Life

N-nitrosadiphenylamine

For freshwater aquatic life, no criterion for N-nitrosodiphenylamine can be derived using the Guidelines, and there are insufficient data to estimate a criterion using other procedures.

For saltwater aquatic life, no criterion for N-nitrosodiphenylamine can be derived using the Guidelines, and there are insufficient data to estimate a criterion using other procedures.

Human Health

N-nitrosodimethylamine

For the maximum protection of human health from the potential carcinogenic effects of exposure to N-nitrosodimethylamine through ingestion of water and contaminated aquatic organisms, the ambient water concentration is zero. Concentrations of N-nitrosodimethylamine estimated to result in additional lifetime cancer risks ranging from no additional risk to an additional risk of 1 in 100,000 are presented in the Criterion Formulation section of this document. The Agency is considering setting criteria at an interim target risk level in the range of 10^{-5}, 10^{-6}, or 10^{-7} with corresponding criteria of 0.026 µg/l, 0.0026 µg/l, and 0.00026 µg/l, respectively.

N-nitrosidiethylamine

For the maximum protection of human health from the potential carcinogenic effects of exposure to N-nitrosodiethylamine through ingestion of water and contaminated aquatic organisms, the ambient water concentration is zero. Concentrations of N-nitrosodiethylamine estimated to result in additional lifetime cancer risks ranging from no additional risk to an additional risk of 1 in 100,000 are presented in the Criterion Formulation section of this document. The Agency is considering setting criteria at an interim target risk level in the range aof 10^{-5}, 10^{-6}, or 10^{-7} with corresponding criteria of 0.0092 µg/1, 0.00092 µg/1, and 0.000092 µg/1, respectively.

N-nitrosodi-n-butylamine

For the maximum protection of human health from the potential carcinogenic effects of exposure to N-nitrosodi-n-butylamine through ingestion of water and contaminated aquatic organisms, the ambient water concentration is zero. Concentrations of N-nitrosodi-n-butylamine estimated to result in additional lifetime cancer risks ranging from no additional risk to an additional risk of 1 in 100,000 are presented in the Criterion Formulation section of this document. The Agency is considering setting criteria at an interim target risk level in the range of 10^{-5}, 10^{-6}, or 10^{-7} with corresponding criteria of 0.013 µg/1, 0.0013 µg/1, and 0.00013 µg/1, respectively.

N-nitrosopyrrolidine

For the maximum protection of human health from the potential carcinogenic effects of exposure to N-nitrosopyrrolidine through ingestion of water and contaminated aquatic organisms, the ambient water concentration is zero. Concentrations of N-nitrosopyrrolidine estimated to result in additional lifetime cancer risks ranging from no additional risk to an additional risk of 1 in 100,000 are presented in the Criterion Formulation section of this document. The Agency is considering setting criteria at an interim target risk level in the range of 10^{-5}, 10^{-6}, or 10^{-7} with corresponding criteria of 0.11 µg/l, 0.011 µg/l, and 0.0011 µg/l, respectively.

Introduction

The nitrosamines belong to a large group of chemicals generally called N-nitroso compounds. Also included in this group are the structurally-related nitrosamides. Because they frequently coexist with N-nitrosamines in the environment, nitrosamides are addressed also in this document.

Synthetic production of N-nitrosamines is limited to small quantities, and the only nitrosamine produced in quantities greater than 450 kg per year is N-nitrosodiphenylamine. It is used as a vulcanizing retarder in rubber processing and in the manufacture of pesticides. Other N-nitroso compounds are produced primarily as research chemicals and not for commerical purposes (U.S. EPA, 1976).

Nitrosamines are characterized by the functional group $-\overset{|}{N}-N=O$ and nitrosamides are characterized by the functional group $-\overset{|}{\underset{O}{\overset{\|}{C}}}-N-N=O$. Depending on the nature of the radical group, nitrosamines exist in several forms, including symmetrical dialkyl-nitrosamines, asymmetrical dialkyl-nitrosamines, nitrosamines with functional groups, cyclic nitrosamines and acyalkylnitrosamines with functional groups, cyclic nitrosamines and acylalkylnitrosamines or nitrosamides (Searle, 1973).

The nitrosamines vary widely in their physical properties and may exist as solids, liquids, or gases. They are soluble in water and organic solvents. Nitrosamines of low molecular weight are volatile at room temperature, and high molecular weight nitrosamines are steam volatile (U.S. EPA, 1976). N-nitrosamines are widespread in the environment, and anthropogenic sources contribute negligibly to these levels.

The most significant source of N-nitrosamines and N-nitrosamides in the environment is probably nitrosation of amine and amide precursors (Bogovski, et al. 1972). These reactions may occur in air, soil, water, food, and animal systems, when the precursors occur simultaneously (Mysliwy, et al. 1974; Fine, et al. 1977b; Rounbehler, et al. 1977; Mills, 1976). Concentrations in the nanogram to microgram per unit volume or mass range have been recorded in air, water, soil, plants, and foodstuffs (Fine, et al. 1977a). Laboratory tests show that N-nitroso-diphenylamine bioconcentrates in the bluegill sunfish by a factor of 217 (U.S. EPA, 1975). A survey of U.S. waters and industrial effluents, conducted by U.S. EPA (1977), recorded levels of various nitrosamines ranging from less than 0.1 µg/l in industrial pipe sources to less than 10 µg/l in ambient streams. The extent of exposure of the general population of N-nitrosamines and N-nitrosamides is unknown. The most significant exposures, resulting from anthropogenic sources, are probably restricted to limited industrial areas (Fine, et al. 1977a, 1977b, 1977c).

Nearly 70 percent of all N-nitrosamines studied have been found to be carcinogenic in a wide range of laboratory animals including various aquatic organisms (U.S. EPA, 1976). The carcinogenic and toxic properties are well established; and both N-nitrosamines and N-nitrosamides are considered to be among the most potent of all mutagenic, teratogenic, and carcinogenic agents known (Montesano and Bartsch, 1976: Czygan et al. 1973). They have been demonstrated to induce

tumors in essentially all vital organs via all routes of administration (Druckrey, 1967). Since tumor production can occur after long-term exposure to small doses, there is concern about human exposure to these agents in water (Druckrey, 1973a, 1973b; Magee, et al. 1976; Lijinsky and Taylor, 1977).

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AQUATIC LIFE TOXICOLOGY*

FRESHWATER ORGANISMS

Introduction

The data base is limited to three fish and invertebrate species; acute tests with N-nitrosodiphenylamine were conducted using static and unmeasured procedures. Feeding studies with dimethylnitrosamaine (N-nitrosodimethylamine) and rainbow trout demonstrated a dose-related carcinogenic response. This response is similar to dose-related effects with mammals and numerous nitrosamines, including dimethylnitrosamaine. Details of these later studies are available in the human health effects portion of this document.

Acute Toxicity

The adjusted 96-hour LC50 for the bluegill is 3,200 µg/l. This value results in a Final Fish Acute Value of 820 µg/l (Table 1). This result is significantly different from that for the mummichog, a saltwater species, for which the adjusted 96-hour LC50 is 1,804,100 µg/l (Table 6). No explanation for this difference can be offered without conjecture.

*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life [43 FR 21506 (May 18, 1978) and 43 FR 29028 (July 5, 1978)] and the Methodology Document in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are the calculations for deriving various measures of toxicity as described in the Guidelines.

The 48-hour EC50 for Daphnia magna after adjustment for test methods is 6,570 µg/l. Since the Guidelines sensitivity factor for invertebrate species is much larger than that for fish species, the Final Acute Value is based on Daphnia magna and is 310 µg/l (Table 2).

Chronic Toxicity

The chronic effects of N-nitrosodiphenylamine on Daphnia magna have been studied (U.S. EPA, 1978) but no adverse effects were observed at any test concentration, even the lowest at 48 µg/l (Table 3). This result divided by the Guidelines species sensitivity factor of 5.1 results in a Final Invertebrate Chronic Value of less than 9.4 µg/l, which also becomes the Final Chronic Value.

Residues

Bioconcentration of N-Nitrosodiphenylamine by the bluegill (U.S. EPA, 1978) reached equilibrium within 14 days and the bioconcentration factor was 217 (Table 4). The depuration rate was rapid so that the half-life of this compound in the tissues was less than 1 day and for this reason N-nitrosodiphenylamine is less likely to be a potential residue hazard for wildlife and consumers of aquatic life.

Miscellaneous

Grieco, et al. (1978) fed Shasta strain rainbow trout dimethylnitrosamine (N-nitrosodimethylamine) in the diet for 52 weeks (Table 5). After this time the fish were placed on a control diet for an additional 26 weeks. No hepatocellular carcinomas were detected at 26 weeks after feeding

began. At 52 weeks, however, a direct dose-related response of hepatocellular carcinoma occurred in trout fed 200, 400, and 800 mg dimethylnitrosamine/kg. A greater incidence of carcinoma was observed at 78 weeks, even though feeding was discontinued after 52 weeks. For further information and details on mammalian carcinogenesis of nitrosamines, the reader is referred to the human health effects portion of this document.

CRITERION FORMULATION

Freshwater - Aquatic Life

Summary of Available Data

The concentrations below have been rounded to two significant figures.

N-nitrosodiphenylamine

Final Fish Acute Value = 820 µg/l

Final Invertebrate Acute Value = 310 µg/l

Final Acute Value = 310 µg/l

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = less than 9.4 µg/l

Final Plant Value = not available

Residue Limited Toxicant Concentration = not available

Final Chronic Value = less than 9.4 µg/l

0.44 x Final Acute Value = 140 µg/l

No freshwater criterion can be derived for N-nitrosodiphenylamine using the Guidelines because no Final Chronic Value for either fish or invertebrate species or a good substitute for either value is available, and there are insufficient data to estimate a criterion. However, the results of the incomplete chronic test with Daphnia magna indicate that the Final Chronic Value would be lower than 9.4 µg/l.

Table 1. Freshwater fish acute values for nitrosamines (U.S. EPA, 1978)

Organism	Bioassay Method*	Test Conc.**	Chemical Description	Time (hrs)	LC50 (ug/l)	Adjusted LC50 (ug/l)
Bluegill, Lepomis macrochirus	S	U	N-Nitroso-diphenylamine	96	5,850	3,200

* S = static

** U = unmeasured

Geometric mean of adjusted values: N-Nitrosodiphenylamine = 3,200 µg/l $\frac{3,200}{3.9}$ = 820 µg/l

Table 2. Freshwater invertebrate acute values for nitrosamines (U.S. EPA, 1978)

Organism	Bioassay Method*	Test Conc.**	Chemical Description	Time (hrs)	LC50 (ug/l)	Adjusted LC50 (ug/l)
Cladoceran, Daphnia magna	S	U	N-Nitroso-diphenylamine	48	7,760	6,570

* S = static

** U = unmeasured

Geometric mean of adjusted values: N-Nitrosodiphenylamine = 6,570 µg/l $\frac{6,570}{21}$ = 310 µg/l

Table 3. Freshwater invertebrate chronic values for nitrosamines (U.S. EPA, 1978)

Organism	Test*	Limits (ug/l)	Chronic Value (ug/l)
	N-Nitrosodiphenylamine		
Cladoceran, Daphnia magna	LC	<48	<48

* LC = life cycle or partial life cycle

Geometric mean of chronic values = <48 µg/l $\frac{<48}{5.1}$ = <9.4 µg/l

Lowest chronic value = <48 µg/l

Table 4. Freshwater residues for nitrosamines (U.S. EPA, 1978)

Organism	Bioconcentration Factor	Time (days)
	N-Nitrosodiphenylamine	
Bluegill, *Lepomis macrochirus*	217	14

Table 5. Other freshwater data for nitrosamines (Grieco, et al. 1978)*

Organism	Test Duration	Effect	Result ug/l
Rainbow trout, Salmo gairdneri	26-78 wks	Dose related hepatocellular carcinomas	Feeding in diet at 200-800 mg/kg

* Data are for dimethylnitrosamine (N-nitrosodimethylamine).

SALTWATER ORGANISMS

Introduction

The only datum for nitrosamines and saltwater aquatic life is the acute effect of N-nitrosodiphenylamine on the mummichog.

Acute Toxicity

When the 96-hour LC50 for the mummichog is adjusted by the Guidelines factors to account for testing methods and species sensitivity, the resultant Final Fish Acute Value for N-nitrosodiphenylamine is 490,000 µg/l. This concentration also becomes the Final Acute Value.

As discussed in the Freshwater Organisms section, this result is significantly different from that derived from data for the bluegill (Final Fish Acute Value = 820 µg/l). No explanation for this difference can be offered without conjecture.

CRITERION FORMULATION

Saltwater - Aquatic Life

Summary of Available Data

The concentrations below have been rounded to two significant figures.

N-nitrosodiphenylamine

Final Fish Acute Value = 490,000 µg/l

Final Invertebrate Acute Value = not available

Final Acute Value = 490,000 µg/l

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = not available

Final Plant Value = not available

Residue Limited Toxicant Concentration = not available

Final Chronic Value = not available

0.44 x Final Acute Value = 220,000 µg/l

No saltwater criterion can be derived for N-nitrosodiphenylamine using the Guidelines because no Final Chronic Value for either fish or invertebrate species or a good substitute for either value is available, and there are insufficient data to estimate a criterion using other procedures.

Table 6. Marine fish acute values for nitrosamines (Ferraro, et al. 1977)

Organism	Bioassay Method*	Test Conc.**	Chemical Description	Time (hrs)	LC50 (ug/l)	Adjusted LC50 (ug/l)
Mummichog, Fundulus heteroclitus	S	U	N-nitroso-dimethylamine	96	3,300,000	1,804,100

* S = static

** U = unmeasured

Geometric mean of adjusted values = 1,804,100 ug/l $\frac{1,804,100}{3.7}$ = 490,000 ug/l

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NITROSAMINES

Human Health Effects

INTRODUCTION

The N-nitrosamines represent one group of those organic compounds characterized by containing a nitroso group (-N=O) attached to a nitrogen (N-nitroso compounds). Closely related to the N-nitrosamines are the N-nitrosamides. The formation of both groups of compounds from precursors in the environment, and in the animal or human body, occurs through a common mechanism (nitrosation). Both groups of compounds are typically highly toxic, again probably through common mechanisms. It is extremely unlikely that the human population would be exposed only to nitrosamines or only to nitrosamides since the precursors of both generally occur together. Thus, although this document is intended to refer specifically to N-nitrosamines, it has been considered prudent to follow the precedent of earlier literature (in which the term "nitrosamines" is frequently used synonymously with N-nitroso compounds) and to include some discussion of the N-nitrosamides.

It has also not proven possible to treat the health effects of N-nitrosamines without considering sources of both preformed N-nitrosamines and their precursors.

SOURCES OF AND ROUTES OF EXPOSURE TO N-NITROSO COMPOUNDS

Exogenous Sources. N-nitrosamines are widespread in the environment. Concentrations in the nanogram to microgram per unit volume or mass range have been recorded in air, water, soil, plants and foodstuffs (Fine, et al. 1977a). Synthetic production is limited to small quantities; N-nitrosodiphenylamine is the only nitrosamine produced in quantities greater than 450 kg per year. Other N-nitroso compounds are produced primarily as research chemicals and not for commercial purposes (Walker, et al. 1976). The most probable source of environmental N-nitrosamines and N-nitrosamides is nitrosation of amine and amide precursors (Bogovski, et al. 1972).

Both nitrosating agents and nitrosatable compounds are ubiquitous in the environment from natural and man-made sources. The form of inorganic nitrogen most widespread is nitrate. Nitrate is a common constituent of plants and is the primary form which plants absorb from the soil. Nitrite is found only in low concentrations because of its greater reactivity. However, nitrate is readily converted to nitrite by microbial reduction, and, according to some evidence (Klubes and Jonsdorf, 1971), bacteria are capable of promoting the synthesis of nitrosamines from a secondary amine and nitrate without conversion of the latter to nitrite. Oxides of nitrogen may also act as nitrosating agents. It has been estimated that 20.7 x 10^9 kg of nitrogen oxides were emitted from industrial, commercial, and domestic sources in the United States during 1970 (U.S.EPA, 1977).

Nitrosatable compounds occur in great variety. Some are ubiquitous in nature either as components of living organisms (for example, amino acids

such as proline, tryptophan and arginine; cyclic amines such as purines and pyrimidines) or as products of the anaerobic decay of protein-rich organic matter (amines, ureas, etc.). Many agricultural chemicals are nitrosatable amino compounds (for example, the antisuckering agent, dimethyldodecylamine; the methylcarbamate insecticides). Amines are emitted from coking plants and petroleum refineries and, together with other forms of combined nitrogen, including nitrates, from sewage treatment plants, etc. Industrial amine production has been reviewed and summarized by Walker, et al (1976).

Nitrosation of amide or amine precursors may occur in the air, soil, water and in some stored or preserved foods. The major requirement is probably the simultaneous presence of precursors (Mills, 1976).

Endogenous Sources. There is now conclusive evidence that nitrosation of amines and amides even in trace concentrations occurs in the gastrointestinal tract of both animals and man (Mysliwy, et al. 1974; Fine, et al. 1977b, Rounbehler, et al. 1977).

Nitrite may be ingested in the food, mainly as a preservative in cured meats. It can originate in the body from reduction of nitrate by bacteria containing the enzyme nitrate reductase. The major site is the oral cavity by bacterial reduction of nitrate in ductal saliva (Tannenbaum, et al. 1974), although other sites have been demonstrated or proposed, including the stomach, in human subjects with gastric hypoacidity (Sander and Schweinsberg, 1972), and the infected urinary bladder (Hawksworth and Hill, 1974). Recent studies (Tannenbaum, et al. 1978a) indicate that nitrite is also formed de novo in the upper portion of the human intestine, probably from

ammonia or organic nitrogen compounds. As material passes through the intestine, some nitrite is converted to nitrate. Absorbed nitrate is recycled into saliva via the salivary glands, the stomach via the parietal glands, and the bladder via the urine. Absorbed nitrite is rapidly destroyed in the blood.

The amount of nitrosamine formed at any site is affected by many factors such as nucleophilicity of the amine, substrate concentration and pH. A detailed discussion is provided by Mirvish (1975). Conditions in the stomach of monogastric animals following a meal (pH range 1 to 5) particularly favor nitrosation. Tannenbaum, et al. (1978a) suggest that nitrite originating in the intestine may react to form N-nitroso compounds in the cecum and colon, which are relatively more acidic than the small intestine. Nitrosamine formation has also been shown to be possible in saliva even at neutral pH, although the amount formed is small (Tannenbaum, et al. 1978b). Some substances, such as thiocyanate, increase the rate of nitrosamine formation (Boyland, et al. 1971). Thiocyanate occurs in saliva, especially that of smokers, and in gastric juice. Others, such as ascorbic acid inhibit the reaction (Mirvish, et al. 1972).

The situation with regard to inhaled potential nitrosamine precursors is considerably more speculative. Nitrous acid is rapidly formed when a mixture of nitric oxide (NO), nitrogen dioxide (NO_2) and water interact in systems of high surface to volume ratio (Wayne, et al. 1951; Graham, et al. 1972; Chan, et al. 1976). It therefore seems reasonable to expect that if these gases are inhaled as pollutants of ambient air, they will rapidly

equilibrate in the lung to form nitrous acid. The neutral, buffered pH of the lung is not normally regarded as favorable to formation of N-nitroso compounds (although, as indicated above, nitrosamine formation in saliva has been observed at neutral pH). However, it has been suggested (U.S.EPA, 1976) that, if nitric acid, sulfuric acid, or other common atmospheric acidic pollutants were inhaled in sufficient amount to produce local acidity within the respiratory tract, nitrosation could occur by interaction between inhaled nitrogen oxides and tissue amines and amides. It is also said (U.S.EPA, 1976) to be theoretically possible for all the precursors necessary for nitrosamine formation to be generated in acid aerosol droplets in an atmosphere containing significant amounts of nitrogen oxides, sulfur oxides and ammonium ion.

It is evident that the human population is exposed to both preformed N-nitroso compounds in the environment and to similar compounds formed endogenously from precursors in the environment. Assessment of the relative significance of various exposure pathways is clearly invalid unless both "nitrosamines" and their precursors are considered.

Exposure through Drinking Water. Precursor chemicals of nitrosamines are ubiquitous in soils and water. The concentration of simple aliphatic amines is normally low (nanogram-to-milligram per kilogram amounts) since they are rapidly metabolized by microorganisms (National Academy of Sciences, 1978). Many pesticides have been shown to be nitrosatable and some, such as atrazine, are only slowly degraded and persist in soil and water. Nitrite concentrations in soil and water are normally low ($\leq$ 1 mg/kg nitrite N). However, the concentrations of nitrite (and its precursors,

ammonia and nitrate) and nitrosatable compounds can be much greater in soils heavily fertilized with organic waste matter or in waters receiving runoff from agricultural areas or discharges of industrial or municipal wastewater containing substantial amounts of amines. Levels of nitrate in municipal drinking waters in the United States seldom exceed 10 mg/l nitrate N although some smaller water supplies and private wells contain much more nitrate. Concentrations as high as 100 to 500 mg/l of nitrate N have been reported in polluted wells (National Academy of Sciences, 1977).

It has been amply demonstrated that nitrosamines are formed in soils, water and sewage after addition of relatively large amounts of secondary or tertiary amines and nitrite or nitrate (Ayanaba, et al. 1973; Ayanaba and Alexander, 1974). N-nitrosodimethylamine has been found in a number of soil samples (Fine, et al. 1977c) at the 1 to 8 µg/kg (dry basis) level. Fine, et al. speculate that this may have arisen from absorption of preformed nitrosodimethylamine from the air or absorption of dimethylamine with subsequent nitrosation. Another possible source is pesticide application. Several pesticides (carbamates and N, N-disubstituted amides) have been shown to yield nitrosodimethylamine upon nitrosation (Mirvish, 1975). Others, such as the phenoxyacetic acid derivatives, are formulated as amine salts; some commercial preparations have been found to contain as much as 0.06 percent nitrosodimethylamine as a contaminant (Fine, et al. 1977a). Nitrosamines are readily leached through the soil profile by percolating water and thus may eventually contaminate surface and ground waters if formed in the soil (Dean-Raymond and Alexander, 1976). These authors have also found N-nitroso diemthylamine to be taken up from soil by spinach and lettuce, the percentage taken up from the soil varying from 0.02

to 5.1 with the experimental conditions. However, under natural conditions, nitrosamines are not commonly found in plants (see below).

Significant concentrations of nitrosamines have been reported for a limited number of samples of ocean water, river water, and waste treatment plant effluent adjacent to or receiving wastewater from industries using nitrosamines or secondary amines in production operations. Thus nitrosodimethylamine has been reported at the 3 to 4 µg/l level in waste water samples (Fine, et al. 1977c). To what extent the nitrosamine arose from impurities in the amine process or from nitrosation in the waste treatment plant is not known. In water samples from wells characterized by both high nitrate levels and coliform counts, the concentration of volatile and non-volatile nonionic nitrosamines was less than 0.015 µg/l (U.S. EPA, 1977). Volatile nitrosamines have not been detected in drinking water (Fine, et al. 1975). However, non-volatile nitrosamines (including N-nitrosatrazine) have been tentatively found in New Orleans water at levels of 0.1 to 0.5 µg/l (Fine, et al. 1976).

Nitrosamines are rapidly decomposed by photolysis and do not persist for a significant time in water illuminated in sunlight. Thus, it is unlikely that they will be present in high (greater than 1 mg/l) concentrations in surface waters. However, in the absence of light they can be expected to persist (Tate and Alexander, 1976). No degradation of N-nitrosodimethylamine, N-nitrosodiethylamine or N-nitrosodipropylamine was observed in lake water during a 3.5 month period (Tate and Alexander, 1975). Fine, et al. (1977a) have shown that nitrosodimethylamines can exist for extended periods of time in the aquatic environment.

Exposure through Food. Many food constituents are either directly capable of conversion to N-nitroso compounds or give rise through chemical action or metabolic processes to nitrosatable products. Walters (1977) has listed some of these compounds. Amino acids such as proline, hydroxyproline, tryptophan, arginine, etc., are nitrosatable. The action of heat on other amino acids can give rise to degradation products such as pipecolic acid containing secondary amino groups. There is no evidence that proteins are nitrosated directly, but they release nitrosatable amino acids during food processing or digestion. Walters suggests that prolyl peptides may be more readily nitrosated than proline itself. A number of other tissue components, such as choline and phospholipids, contain tertiary amines and quaternary ammonium which can be dealkylated to secondary amines. Many of the purine and pyrimidine bases of the nucleic acids contain amino groups capable of forming N-nitroso derivatives, as do some vitamins, for example folic acid. Other nitrosatable compounds include caffeine in coffee, theanine in tea, and orotic acid in milk. Some pesticides (for example, atrazine, carbaryl, ferbam, simazin) are nitrosatable and hence residues in or on food represent another source of precursors of N-nitroso compounds (Elsperu and Lijinsky, 1973).

Nitrate and nitrite are also well supplied in the diet. The mean intake in food of nitrate plus nitrite in the United States has been calculated to be approximately 120 mg per day (White, 1975), although there must be considerable individual variability. According to these estimates 86 percent of the nitrate comes from vegetables such as celery, potatoes, lettuce, melons, cabbage, spinach and root vegetables; some, such as spinach and beets, contain 2000-3000 ppm of nitrate. Cured meat supplies

9 percent of the nitrate. Only 2 percent of the nitrite is supplied by vegetables; 21 percent comes from cured meat.

Nitrate is secreted in the saliva, the mean amount being approximately 40 mg per day. Of this, about 10 mg per day is reduced to nitrite in the mouth by the oral flora (Tannenbaum, et al. 1974). These quantities, although internally derived, also represent inputs to the gastrointestinal tract. Ingestion of vegetables containing high levels of nitrate has been shown to lead to extremely high concentrations of nitrite in saliva, and these levels may persist for several hours (Tannenbaum, et al. 1976).

Preformed nitrosamines have been found in food, particularly in meats such as sausages, ham and bacon which have been cured with nitrite. To date, analyses have been confined largely to the volatile N-nitroso compounds. N-nitrosodimethylamine has been found to be present in a variety of foods (including smoked, dried or salted fish, cheese, salami, frankfurters and cured meats) in the 1 to 100 μg/kg range, but more usually in the 1 to 10 μg/kg range (Montesano and Bartsch, 1976). Other nitrosamines tentatively identified in meat products are N-nitrosodiethylamine, N-nitrosopiperidine and N-nitrosopyrrolidine (Montesano and Bartsch, 1976). N-nitrosopyrrolidine has been consistently found to be present in cooked bacon at the 10 to 50 μg/kg concentration level but not in raw bacon (Fine, et al. 1977a). It apparently arises from N-nitrosoproline by decarboxylation during the cooking process (Lijinsky, et al. 1970). The source of nitrosamines in meat products is undoubtedly nitrosation; a report from a USDA Expert Panel on Nitrites and Nitrosamines (U.S. Dep. Agric., 1978)

therefore recommends substantial reductions in the amounts of nitrate and nitrite used in cured meats.

Nitrosamines are not commonly found in plants. N-nitrosodimethylamine is said to have been isolated from the fruit Solanum incanum, used by the Transkeian Bantu to sour milk (DuPlessis, et al. 1969). Nitrosamines have also been reported in mushrooms in concentrations of 0.4 to 30 µg/kg (Ender and Ceh, 1968) and in wheat plants, grain and flour (Hedler and Marquardt, 1968). However, Thewlis (1968) was unable to find N-nitrosodiethylamine in wheat flour and Kroller (1967) also was unable to detect N-nitrosodiethylamine at levels greater than 10 µg/kg in only one of 30 samples of wheat flour examined.

It is necessary to note that studies prior to 1970 reporting the presence of nitrosamines in foods are open to question since the analytical methodology employed is now known to have been non-specific.

A bioconcentration factor (BCF) relates the concentration of a chemical in water to the concentration in aquatic organisms, but BCF's are not available for the edible portions of all four major groups of aquatic organisms consumed in the United States. Since data indicate that the BCF for lipid-soluble compounds is proportional to percent lipids, BCF's can be adjusted to edible portions using data on percent lipids and the amounts of various species consumed by Americans. A recent survey on fish and shellfish consumption in the United States (Cordle, et al. 1978) found that the per capita consumption is 18.7 g/day. From the data on the nineteen major species identified in the survey and data on the fat content of the edible portion of these species (Sidwell, et al. 1974), the relative consumption of the four major groups and the weighted average percent lipids for each group can be calculated:

Group	Consumption (Percent)	Weighted Average Percent Lipids
Freshwater fishes	12	4.8
Saltwater fishes	61	2.3
Saltwater molluscs	9	1.2
Saltwater decapods	18	1.2

Using the percentages for consumption and lipids for each of these groups, the weighted average percent lipids is 2.3 for consumed fish and shellfish.

A measured steady-state bioconcentration factor of 217 was obtained for N-Nitrosodiphenylamine using bluegills containing about one percent lipids (U.S. EPA, 1978). An adjustment factor of 2.3/1.0 = 2.3 can be used to adjust the measured BCF from the 1.0 percent lipids of the bluegill to the 2.3 percent lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for N-Nitrosodiphenylamine and the edible portion of all aquatic organisms consumed by Americans is calculated to be 217 x 2.3 = 500.

Exposure through Ambient Air. In theory there are several possible routes to the formation of nitrosamines in the atmosphere. These have been discussed in some detail (U.S.EPA, 1976, 1977). Due to the photolabile nature of nitrosamines it seems unlikely that concentrations in ambient air would exceed a few ppb except very near sources of direct emissions of nitrosamines. This has since been confirmed by recent observations of Fine, et al. (1977a). N-nitrosodimethylamine was identified as an air pollutant near two chemical plants, one using the amine as a raw material and the other discharging it as an unwanted byproduct. Typical levels at the first factory were 6 to 36 $\mu g/m^3$ on site, 1 $\mu g/m^3$ in the residential neigh-

borhood adjacent to the factory, and 0.1 $\mu g/m^3$ 2 miles away. Typical daily human exposures were calculated to be 39 μg on site, 10 μg in the adjacent residential neighborhood, and 0.3 μg 2 miles away. Typical levels adjacent to the second site were 0.001 to 0.04 $\mu g/m^3$. However, nitrosamines were detected only twice at 40 collection points in New Jersey and New York City, and then only below the 0.01 $\mu g/m^3$ level. Fine, et al. (1977a) conclude that airborne N-nitroso compounds may not represent a daily widespread air pollution problem, but rather a localized problem associated with a particular segment of a specialized industry or with a particularly severe pollution level.

Other Sources and Routes of Exposure. Many drugs and medicines contain secondary or tertiary amine groups. Model and animal experiments have demonstrated that these compounds can be readily nitrosated and thus suggest that they are precursors of N-nitroso compounds *in vivo* (Lijinsky and Taylor, 1977).

Tobacco and tobacco smoke contain both secondary amines and nitrosamines. Nitrosamines are not present in fresh tobacco, but are found during curing (Hoffman, et al. 1974). In relatively high concentrations (in the order of 100 mg/m^3) secondary amines and nitrogen dioxide can react rapidly to form nitrosamines; this reaction apparently occurs in tobacco smoke (U.S.EPA, 1977). The mainstream smoke from an 85 mm U.S. blended cigarette without a filter tip has been found to contain 0.084 μg N-nitrosodimethylamine, 0.030 ug N-nitrosomethylethylamine, 0-137 μg N-nitrosonornicotine, and traces of N-nitrosodiethylamine (Hoffman, et al.

1974). It can be estimated that the intake from smoking 20 cigarettes per day would therefore be approximately 2 μg N-nitrosodimethylamine, 1 μg N-nitrosomethylethylamine, and 3 μg N-nitrosonornicotine. Walker, et al. (1976) have attempted to evaluate the exposure to nitrosamines of a non-smoker exposed to tobacco smoke. Assuming exposure to the smoke of five simultaneously burning cigarettes under crowded conditions with no ventilation, levels in air were calculated to be approximately 0.015 $\mu g/m^3$ N-nitrosodimethylamine, 0.004 $\mu g/m^3$ N-nitrosomethylethylamine and 0.015 $\mu g/m^3$ N-nitrosonornicotine, with traces of N-nitrosodiethylamine.

N-nitroso-bis(2-hydroxyethyl)amine (N-nitrosodiethanolamine) has been reported to occur in cosmetic preparations, including facial creams, hand lotions and hair shampoos, in concentrations ranging from 20 to 48,000 μg/kg (Fan, et al. 1977). The extent to which this compound is absorbed from the skin is unknown.

Commercial pesticide formulations available for home use have been found to contain as much as 0.06 percent N-nitrosodimethylamine as a contaminant (Fine, et al. 1977a). The contamination could have arisen during the manufacturing process or from nitrosation of dimethylamine by nitrite rust inhibitors added to prevent corrosion of the can. The main routes of exposure from home use of pesticides can be expected to be inhalation and absorption through the skin during spraying operations. Severn (1977), using data from three studies on inhalation and dermal exposure to pesticides during spraying of orchards, estimated that the intake from skin deposition, assuming 50 percent absorption, averaged about 325 times more

than the intake via inhalation and concluded that the same ratio would hold for individuals performing hand spot-spraying. Inhalation, dermal, and/or oral exposure could also occur from careless use of these pesticides.

Relative Significance of Routes of Exposure. Fine, et al. (1977a) have calculated the daily exposure to preformed N-nitrosamines under worst case conditions (Table 1). The intake from nitrite preserved foods assumes 100 g cooked bacon to be consumed daily. Air exposure is based on the highest concenrations measured on a factory site. For the general population, however, exposure information is very limited. It has been estimated

Table 1. Calculated daily human exposure to N-nitroso compounds (Fine, et al. 1977a)

	Daily intake (µg)				
	Nitrosodimethylamine + Nitrosodiethylamine		Nitrosopyrrolidine	Nitrosonornicotine	Other
Nitrite preserved foods, 100 g.	1		5		
Tobacco smoke, 20 cigarettes	2			3	
Drinking water, New Orleans					8*
Air, factory site	40				10*
Herbicide formulation, 1 ml spill	640				

* Tentative, unconfirmed identification as N-nitroso compound.

that air, diet and smoking all play a roughly equivalent role in direct human exposure, contributing a few micrograms per day, with direct intake from drinking water probably much less than 1 μg per day (U.S.EPA, 1976).

There is even greater uncertainty with regard to the significance of exposure to precursors. The chief source of the nitrate body burden, except in the newborn, is ingested vegetables, unless rural well water high in nitrate is consumed. Food and water normally contribute a few hundred milligrams per day. Inhalation may also contribute several

Table 2. Calculated average daily exposure to nitrate and nitrite of U.S. residents (White, 1975)

Source	Nitrate		Nitrite	
	mg	%	mg	%
Vegetables	86.1	86.3	0.20	1.8
Fruits, juices	1.4	1.4	0.00	0.0
Milk and products	0.2	0.2	0.00	0.0
Bread	2.0	2.0	0.02	0.2
Water	0.7	0.7	0.00	0.0
Cured meats	9.4	9.4	2.38	21.2
Saliva	(30.0)*		8.62	76.8
Total	99.8	100	11.22	100

*Not included in total.

hundred micrograms per day (U.S.EPA, 1977). On a daily basis, the major source of nitrite is saliva (Table 2). However, salivary nitrite is presented to the body as a continuous, low-level input, in comparison with the relatively high concentrations over short periods resulting from ingestion of cured meats. This may be significant since the rate of nitrosation is a function of the square of the nitrite concentration (U.S.EPA, 1977). Estimates of the contribution to the daily intake of N-nitroso compounds (as nitrosodimethylamine) have been attempted (Natl. Acad. Sci., 1978). Using blood levels of nitrosamines measured in one human subject before and after consuming a lunch consisting of spinach, cooked bacon, tomato, bread and beer (Fine, 1977b), it was calculated that in vivo formation contributed 2.8 μg per day nitrosodimethylamine. For various reasons it is believed that the total amount of nitrosamine formed may have been considerably more than this. A second approach assumed that the rate of formation of nitrosamines is equal to 5 percent of the amount of nitrite present. This yielded an estimated daily production from precursors of 962 μg nitrosodimethylamine. However, this approach is likely to give a substantial overestimate. The conclusion appears inescapable that in vivo nitrosation provides a major contribution to the total body burden of N-nitroso compounds.

It must also be concluded that water supplies are a relatively minor source when compared with other potential sources of either preformed N-nitroso compounds or their precursors.

PHARMACOKINETICS

Following intravenous injection into rats, nitrosamides (e.g., N-nitrosomethylurea, N-nitrosoethylurea) and nitrosamines (e.g., N-nitroso-dimethylamine, N-nitrosomorpholine) are rapidly and apparently fairly uniformly distributed in the body (Magee, 1972; Stewart, et al. 1974). Orally administered nitrosodiethylamine is found in the milk of lactating rats (Schoental, et al. 1974). Both nitrosamides (e.g., nitrosodiethyl-amine) and nitrosamines (e.g., N-nitrosoethylurea) can presumably cross the placenta since they are capable of inducing neoplasms in the offspring if administered to rats in late pregnancy (Magee, et al. 1976).

The nitrosamides are rapidly metabolized in the animal body. The half-lives of intravenously administered N-nitrosomethylurea and N-nitro-soethylurea in rats are about 2 min. and 5-6 min. respectively. The metabolism of ^{14}C-labeled N-methyl-N'-nitro-N-nitroso-guanidine has been studied in some detail. Following an oral dose, most of the radioactivity was excreted in the urine within 24 hours and less than 3% in the feces. Less than 3% of the radioactivity remained in the body as acid-insoluble materials at 24-48 hours (Magee, et al. 1976).

The nitrosamines are metabolized less rapidly and persist in the body unchanged for a longer period. The rate of metabolism depends upon the chemical structure. In the rat or mouse, administration of ^{14}C-labeled nitrosodimethylamine leads to about 60% of the isotope appearing as $^{14}CO_2$ within 12 hours. Corresponding figures for labeled nitrosodiethylamine and

nitrosomorpholine are about 45 percent and 3 percent respectively. For the three compounds, corresponding urinary excretions are 4, 14 and 80 percent, respectively. Metabolic products of dialkylnitrosamines found in the urine include other nitroso compounds, formed by ω-oxidation of the alkyl groups to give the corresponding alcohols and carboxylic acids (Magee, et al. 1976).

In vitro studies have demonstrated that the organs in the rat with the major capacity for metabolism of nitrosodimethylamine are the liver and kidney and that this compound is metabolized to a DNA-methylating agent by human liver slices at a rate slightly slower than but comparable with that of rat liver slices (Montesano and Magee, 1974). (It is the product(s) of metabolism of N-nitrosamines which are thought to be responsible for the mutagenicity and/or carcinogenicity of many of them. One hypothesis is that these active intermediates alkylate DNA at specific sites). Although the liver appears to be the major site of decomposition, other organs, such as kidney and lung, possess varying capacity to metabolize nitrosamines. The relative metabolic activity of different organs toward the same compound varies between species (Magee, et al. 1976).

Evidence to support the various proposed metabolic pathways of N-nitroso compounds is inconclusive. However, the in vitro studies of Montesano and Magee (1974) indicate that nitrosamines are metabolized similarly by human, guinea pig, and rat tissue.

EFFECTS

General Toxicity. N-nitroso compounds are acutely toxic to every animal species tested and are also poisonous to humans.

The dialkyl and cyclic N-nitrosamines are characteristically hepatoxins, producing hemorrhagic centrilobular necrosis. In experimental animals acute exposure to nitrosodimethylamine or nitrosodiethylamine produces liver lesions in 24 to 48 hours; death occurs in 3 to 4 days or the animals survive and apparently recover completely in about 3 weeks. Other organs than the liver are less severely affected; the main features are peritoneal and sometimes pleural exudates, which may contain a high proportion of blood, and a tendency to hemorrhage into the lungs and other organs. Kidney lesions, limited to the convoluted renal tubules, and testicular necrosis have been described in protein-deficient rats following treatment with nitrosodimethylamine (Magee, et al. 1976).

The livers of rats and other species chronically exposed to nitrosamines exhibit various pathological changes, including biliary hyperplasia, fibrosis, modular parenchymal hyperplasia, and the formation of enlarged hepatic parenchmal cells with large nuclei (Magee, et al. 1976). Chronic administration of many nitrosamines induces tumors of the liver and other organs (see below).

The N-nitrosamides also induce a liver necrosis, but it is not as pronounced as that seen with the N-nitrosamines and is localized in the

periportal areas. Unlike the nitrosamines, the nitrosamides cause severe tissue injury at the site of contact. The degree of local damage may be related to the rate at which the compound decomposes at the site since the damage is probably caused by a breakdown product rather than by the compound itself. The systemic targets of the nitrosamides are mainly the organs of rapid cell turnover, including the bone marrow, crypt cells of the small intestine, and lymphoid tissues (Magee, et al. 1976).

The effects of human exposure to nitrosodimethylamine were first reported by Freund in 1937. The following description is from Weisburger and Raineri (1975). "Freund recorded the case of a young chemist engaged in the synthesis of dimethylnitrosamine, who presented with a number of syndromes eventually traced to occupational exposure. The patient had ill-defined pains in the abdomen, exhaustion, headaches, and distended abdomen. A second case, which involved an accidental single severe exposure due to a spill of nitrosamine, again led to abdominal fluid accumulation. During an exploratory laparotomy, ascitic fluid was found and the liver was enlarged. This patient failed to survive. Microscopic findings at autopsy revealed liver necrosis and areas of intense regenerative proliferation of the liver cell."

Further cases are now on record. Of two men accidentally exposed to nitrosodimethylamine used as a solvent in an automobile factory, one recovered after exhibiting signs of liver damage, while the other died in a clinical accident and a necropsy revealed a cirrhotic liver with regenerating nodules. Two of three men in an industrial research laboratory,

working with nitrosodimethylamine over a period of 10 months, showed signs of liver injury. One died of bronchopneumonia and a necropsy found liver cirrhosis. The other developed a hard liver with an irregular surface, but recovered after exposure was terminated (Shank, 1975). The two individuals surviving this 1953 episode were still alive in 1976 (Weisburger and Raineri, 1975).

The acute toxicity of the N-nitroso compounds varies considerably. Single dose oral LD_{50} values in adult rats, range from 18 mg/kg body weight for N-nitrosomethylbenzylamine to more than 7,500 mg/kg for N-nitrosoethyl-2-hydroxy-ethylamine (Table 3). The acute oral LD_{50} in the rat for nitroso-diphenylamine, the only nitrosamine now produced in the U.S. in amounts greater than 450 kg per year, is given as 1,650 mg/kg (Natl. Inst. Occ. Safety Hlth. 1976) or 3,000 mg/kg (Druckrey, et al. 1967). The relationship between structure and acute toxicity is not fully understood; however, for the dialkylnitrosamines acute toxicity appears to decrease with chain length (Shank, 1975). The predominant hepatoxic effect of these compounds is consistent with the hypothesis that the biologically active species is a metabolite and not the parent compound since the liver, metabolically, is generally the most active organ. It is unlikely that under environmental conditions N-nitroso compounds would be present in sufficient quantity to provide an acutely toxic dose.

Mutagenicity. The N-nitroso compounds include some of the most powerful chemical mutagens known. Montesano and Bartsch (1976) reported on the mutagenicity of 90 N-nitroso compounds from direct mutagenicity assays and

Table 3. - Acute oral LD_{50} values (Druckrey, et al. 1967) and relative carcinogenic potency expressed as log $(1/D_{50})$ (Wishnok and Archer, 1976) in BD rats. Classification of of N-nitroso compounds follows that of Druckrey, et al. (1967)

COMPOUND	LD_{50} (mg/kg)	$Log(1/D_{50})$
Symmmetrical dialkyl(aryl)nitrosamines:		
N-nitrosodimethylamine	40	2.27
N-nitrosodiethylamine	280	3.20
N-nitrosodi-n-propylamine	480	2.05
N-nitrosodi-iso-propylamine	850	0.97
N-nitrosodiallylamine*	800	-
N-nitrosodi-n-butylamine	1,200	1.61
N-nitrosodi-n-amylamine	3,000	0.59
N-nitrosodicyclohexylamine*	5,000	-
N-nitrosodiphenylamine*	3,000	-
N-nitrosodibenzylamine*	900	-
Asymmetrical alkyl(aryl)nitrosamines:		
N-nitrosomethylethylamine	90	2.32
N-nitrosomethylvinylamine	24	2.89
N-nitrosomethylallylamine	340	2.10
N-nitrosomethyl-n-amylamine	120	2.60
N-nitrosomethylcyclohexylamine	30	2.98
N-nitrosomethyl-n-heptylamine	-	1.53
N-nitrosomethylphenylamine	280	1.60
N-nitrosomethylbenzylamine	18	3.10
N-nitrosomethyl-(2-phenylethyl)amine	48	3.01
N,N'-dimethyl-N,N'-dinitrosoethylenediamine	150	2.40
N-nitrosoethylvinylamine	88	2.64
N-nitrosoethyl-iso-propylamine	1,100	1.49
N-nitrosoethyl-n-butylamine	380	2.11
N-nitrosoethyl-tert-butylamine*	1,600	-
N-nitroso-n-butyl-n-amylamine	2,500	1.00
Cyclic nitrosamines:		
N-nitrosopyrrolidine	900	1.41
N-nitrosoproline (ethyl ester)*	5,000	-
N-nitrosopiperidine	200	1.91
N,N'-dinitrosopiperazine	160	1.95
N-nitroso-N'-methylpiperazine	1,000	0.95
N-nitroso-N'-carbethoxypiperazine	400	1.91

Table 3. (Continued)

COMPOUND	LD_{50} (mg/kg)	$Log(1/D_{50})$
Cyclic nitrosamines: (Continued)		
N-nitrosoindoline	320	0.88
N-nitrosomorpholine	320	1.95
N-nitrosohexamethyleneimine	340	-
N-nitrosoheptamethyleneimine	280	-
N-nitrosooctamethyleneimine	570	-
N--nitroso compounds with functional substituent groups:		
3-(N-nitroso-N-methylamino)-sulfolane	750	1.82
N-nitroso-N-phenylhydroxylamine	2,000	1.15
N-nitrosotrimethylhydrazine	95	2.24
N-nitrosoethyl-2-hydroxyethylamine	7,500	0.18
N-nitroso-bis(2-hydroxyethyl)amine	7,500	-
N-nitroso-bis(acetoxyethyl)amine	5,000	0.74
N-nitroso-n-butyl-(4-hydroxy-n-butyl)amine	1,800	1.51
N-nitrosomethyl-2-chloroethylamine	22	3.21
N-nitrosomethylcyanomethylamine	45	2.18
N-nitroso-bis(cyanomethyl)amine	163	1.92
N-nitrososarcosine	5,000	0.60
N-nitrosoethylsarcosinate	4,000	1.18
2-methyl-2(N-nitroso-N-methylamine) -pentan-4-one	2,100	1.04
Nitrosamides:		
N,n'-dinitroso-N,N'-dimethyloxamide	96	2.40
N-methyl-N-nitrosoacetamide	20	2.31
N-methyl-N-nitrosourethane	240	2.01
N-ethyl-N-nitrosourethane	-	1.96
N-methyl-N-nitrosourea	110	2.18
N,N'-dimethyl-N-nitrosourea	280	1.95
N-nitrosotrimethylurea	240	2.00
N-ethyl-N-nitrosourea	240	2.67
N-n-butyl-N-nitrosourea	1,200	2.10
Hydrazodicarboxylic acid bis(methyl -nitrosamide)	200	2.38
N-methyl-n'-nitro-N-nitrosoguanidine	420	2.51
N-nitrosoimidazolidone	250	2.26

*Non-carcinogenic in BD rats (Druckrey, et al 1967).

dominant lethal tests. Data on chromosome observations and tests in Drosophila melanogaster are also listed. As with other biological effects, there is a clear distinction between the mutagenic actions of N-nitrosamides and N-nitrosamines. N-nitrosamides are mutagenic in almost all test systems, due to non-enzymic formation of degradation products. N-nitrosamines, on the other hand, are not mutagenic in microbial test systems without metabolic activation.

Liver microsomal preparations from mouse, rat, hamster and man are capable of activating nitrosamines. Czygan, et al. (1973), using human liver microsomes obtained from patients undergoing abdominal surgery, found considerable variations in the capacity of the microsomes to activate the mutagenicity of N-nitrosodimethylamine. The cytochrome P-450 content showed proportional variations. (Cytochrome P-450 is the terminal enzyme in the microsomal system responsible for metabolism of foreign compounds). Czygan, et al. attributed the variations in cytochrome P-450 content to "diseases, therapy, or environmental pollutants." Czygan, et al. (1974) later demonstrated a positive correlation between the protein and choline content of the diet and the microsomal P-450 content, and concluded that activation of nitrosamines can be influenced by nutritional factors. Extracts from organs other than liver are either ineffective or much less effective in activating nitrosamines to bacterial mutagens. Yet these organs may be the target for tumor induction in vivo by the same compounds. Thus, both nitrosodimethylamine and nitrosodiethylamine induce tumors in mouse lung and rat kidney; yet rat, mouse and hamster lung microsomal preparations and mouse kidney preparations are ineffective in

activating those compounds to mutagens in Salmonella typhimurium and Escherichia respectively (Montesano and Bartsch, 1976).

Nitrosodimethylamine and nitrosodiethylamine have been reported to induce forward and reverse mutations in S. typhimurium, E. coli, Neurospora crassa, etc., gene recombination and conversion in Saccharomyces cerevisiae, "recessive lethal mutation" in Dropsophila, and chromosome aberrations in mammalian cells (Montesano and Bartsch, 1976). They gave a negative response in the mouse dominant lethal test, probably due to the inability of the germ cells in the male to metabolize these compounds.

Not all N-nitroso compounds have been found to be mutagenic, although many have been tested only in microbial systems. Of the 23 N-nitrosamines listed by Montesano and Bartsch as having been tested in systems which included metabolic activation, six show no mutagenic activity. These include N-nitrosodiphenylamine, which is reported as yielding a negative response in both S. typhimurium and E. coli after activation with a rat liver microsomal preparation (Bartsch, et al. 1976; Nokajima, et al. 1974).

Teratogenicity. N-nitroso compounds can also be potent teratogens. The effects of experimental administration to pregnant animals have been studied systematically by Druckrey (1973a). In summary, whereas the N-nitrosamides were found to be teratogenic over an extended period of gestation, the N-nitrosamines were active only when administered late in pregnancy. Thus, near-LD_{50} levels of N-nitrosoalkylureas and N-nitroso-

alkylanilines given to pregnant rats on day 9 or 13 of gestation produced malformations of the eye and brain in the offspring: similar levels of N-nitrosodimethylamine or N-nitrosodiethylamine did not (Napalkov and Alexandrov, 1968). Given at other periods of development, both N-nitrosamines and N-nitrosamides have been shown to be embryotoxic or carcinogenic (Druckrey, 1973b).

The two principal factors determining the response appear to be the state of differentiation of the various embryonic tissues and the metabolic competence of these tissues. Magee (1973) has adduced evidence suggesting that the lack of teratogenic and carcinogenic response to N-nitrosamines in early and mid-pregnancy is because the tissues have not yet acquired the competence for metabolic activation.

In Druckrey's studies (1973a), it was observed that some malformations, mainly those of the central and peripheral nervous systems, were associated with good survival times and that no tumors appeared at the sites of malformation. This led Druckrey to suggest that teratogenesis and carcinogenesis are two independent processes and that the molecular mechanisms of induction may be different.

Carcinogenicity

Animal Data: Magee, et al. (1976) summarized data from studies through about 1975 on the carcinogenic activity of N-nitroso and related compounds. Of the 107 N-nitroso compounds (including 83 N-nitrosamines)

listed, 87 (including 67 N-nitrosamines) are reported as having carcinogenic activity. Since that time more compounds have been tested and, to date, approximately 100 N-nitroso compounds are known to be carcinogenic in one or more species of experimental animal (Lijinsky and Taylor, 1977).

All animal species tested are vulnerable. These include mice, rats, Chinese, Syrian and European hamsters, gerbils, guinea pigs, rabbits, mink, dogs, pigs and monkeys. Sensitivity varies with species. The African white-tailed rat, Mystromys albicaudatus, apparently remarkably free from spontaneous tumors, developed liver tumors after treatment with nitrosodiethylamine, although only after about 40 weeks exposure to 50 to 100 mg/l in the drinking water as compared with rats in which extensive hepatocellular carcinomas were seen 10 weeks after a 10-week exposure at 40 mg/l (Yamamoto, et al. 1972). Not all carcinogenic N-nitroso compounds have induced tumors in all species. The cyclic nitrosamine N-nitrosoazetidine (N-nitrosotrimethyleneimine) is reported as inducing lung, liver and kidney tumors in the rat, and lung and liver tumors in the mouse but induced no tumors under the test conditions used in the Syrian golden hamster; toluene-p-sulfonylmethylnitrosamide is said to have failed to induce tumors in the rat but to produce lung tumors in the mouse (Magee, et al. 1976).

Not all N-nitrosamines have been found to induce tumors, although in most cases only one test species has been used, usually the rat. Those compounds observed by Druckrey, et al. (1967) to give a negative response in rats are indicated in Table 3. Others include N-nitrosoethyl-(3-hydroxypropyl)amine, N-nitroso-n-butylcarboxymethylamine, N-nitroso-n-

butyl-(3-hydroxypropyl)amine, N-nitroso-n-butyl-(3-hydroxybutyl)amine, N-nitroso-tert-butyl-(4-hydroxybutyl)amine (Okada, et al. 1976) and guvacoline (Lijinsky and Taylor, 1977). It is interesting to note that apparently all N,N-dialkylnitrosamines containing a tert-butyl group are noncarcinogenic (Heath and Magee, 1962). The list includes N-nitroso-L-proline, found in cured meats, particularly bacon. Although non-carcinogenic itself, nitrosoproline gives rise to the carcinogenic N-nitrosopyrrolidine during cooking (Lijinsky, et al. 1970). N-nitrosodiphenylamine is also reported by a number of investigators as being non-carcinogenic in the rat. However, N-nitrosodiphenylamine and other aromatic nitrosamines are capable of transnitrosation, i.e., under suitable conditions, their nitroso group can be transferred to appropriate amine-type compounds. It is thus possible that non-carcinogenic transnitrosating agents could form new carcinogenic N-nitroso compounds in the stomach (Singer, et al. 1977b).

The carcinogenic N-nitroso compounds are capable of inducing tumors in a wide variety of tissues, many compounds exhibiting a remarkable target organ specificity (organotropism) sometimes modified by the route of administration. Druckrey, et al. (1967) studied the effects of a large number of N-nitroso compounds following prolonged administration to adult rats of the BD strain (said to exhibit a spontaneous malignant tumor rate of 1 percent at 500 days). In general, daily doses were approximately 2.5 percent of the LD_{50} values listed in Table 3, and were administered in drinking water over the lifespan. Pilot experiments used higher dose rates (5 percent or more of the LD_{50}) and some animals received the N-nitroso

compounds by subcutaneous or intravenous injection or inhalation. The mean time to tumor varied, with dose rate and compound, between 160 and 840 days. Druckrey, et al. (1967) made the following general observations (paraphrased from the English summary to their paper).

All symmetrically substituted dialkylnitrosamines produced carcinomas of the liver. The only exception was N-nitrosodi-n-butylamine, which also lead to carcinomas of the urinary bladder. Subcutaneous injection of this compound produced only bladder tumors. N-nitrosodiamylamine given subcutaneously selectively produced lung cancer.

Asymmetrical dialkylnitrosamines, especially those possessing a methyl group and with the second substituent group amyl-, cyclohexyl-, phenyl-, benzyl- or phenylethyl-, and also N,N'-dimethyl-N,N'-dinitrosoethylene diamine, N-nitrosethylvinylamine, and N-nitrosoethyl-n-butylamine, selectively produced carcinomas of the esophagus following both oral and parenteral administration. N-nitrosomethylalkylamines induced maglinant tumors of the kidney, particularly after intravenous injection.

The cyclic nitrosamines, N-nitrosopyrrolidine, N-nitrosomorpholine, and N-nitroso-N'-carbethoxypiperazine induced cancer of the liver. N-nitrosopiperidine and N,N'-dinitrosopiperazine produced carcinomas of the esophagus after both oral and intravenous administration but tumors of the nasal cavity, mostly esthesioneuroepitheliomas, after subcutaneous injection.

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Nitrosamines with functional substituent groups also produced malignant tumors in different organs. 3-(N-nitroso-N-methylamine)-sulfolane and N-nitrososarcosine and its ethyl ester induced esophageal cancer. N-nitroso-n-butyl-(4-hydroxy-n-butyl)amine selectively induced carcinomas of the urinary bladder. N-nitrosoethyl-2-hydroxyethylamine and N-nitroso-bis(2-hydroxyethyl)amine regularly produced liver tumors following chronic exposure but exhibited minimal toxicity in acute experiments (LD_{50}, 7,500 mg/kg).

Several N-nitrosamides produced carcinomas of the fore-stomach after oral administration or local sarcomas at the site of injection. Intravenous N-methyl-N-nitrosourethane selectively produced lung cancer. Methylnitrosoureas induced malignant tumors in the brain, spinal cord, and/or peripheral nervous system.

Lijinsky and his co-workers have systematically studied the effects of modification of chemical structure on the biological activity and organ specificity of the nitrosamines. They have found that minor changes can have a profound effect on the target organ. For example, chronic administration in the drinking water of N-nitrosohexamethyleneimine induces liver tumors in rats; of N-nitrosoheptamethyleneimine, lung tumors. Lijinsky (1977) has discussed his findings in relation to what is known of the mechanism of action of nitrosamines (see below). His conclusion is that the major factor responsible for variations in biological activity is the reactivity of hydrogen atoms on carbon atoms adjacent to the nitroso group (alpha hydrogen atoms).

The response to a particular compound also varies between species. The following attempt to illustrate the diversity of responses is derived from Magee, et al. (1976). In most species, as in the rat, the predominant tumors following prolonged oral administration of dialkyl cyclic and many other N-nitrosamines are in the liver. Tumors in rats have been described as hepatomas and hepatocellular carcinomas, chloangiamas and cholangiocarcinomas, fibrosarcomas, and angiosarcomas. The tumor type(s) observed in mice depend upon both the strain and the compound. Nitrosodimethylamine produced mainly hemangiomatous tumors, with few parenchymal cell tumors. Nitrosodiethylamine induced mainly parenchymal tumors in seven strains of mice but predominantly hemangiosarcomas and hemangioendotheliomas in two strains. Nitrosodiethylamine given to Syrian golden hamsters by the intragastric, intraperitoneal or intradermal routes produced hepatocellular carcinomas which metastasized and were transplantable; continuous oral administration induced cholangiocarcinomas. However, following single or multiple subcutaneous injections both adult and newborn hamsters developed mainly respiratory tumors and very few liver tumors. In the Syrian golden hamster respiratory tract tumors induced by nitrosodiethylamine are confined mainly to the nasal cavities, larynx, and trachea irrespective of the route of administration. In the mouse, guinea pig and rabbit liver tumors following prolonged oral administration of nitrosodiethylamine are accompanied by adenocarcinomas of the lung.

In their first studies demonstrating the carcinogenicity of nitrosodimethylamine, Magee and Barnes (1956) reported that 19 of 20 rats fed 50 mg/kg in the diet continuously developed primary hepatic tumors within 40

weeks. However, they later (Magee and Barnes, 1959) found that, in rats exposed for 1 week at 100 or 200 mg/kg in the diet, kidney tumors predominated over liver tumors. A single, near-LD_{50} dose (30 mg/kg body weight) of nitrosodimethylamine produced no progressive liver lesions nor liver tumors but a 20 percent incidence of kidney tumors. A single intraperitoneal injection given to newborn mice induced hepatocellular carcinomas (Toth, et al. 1964). A single dose to partially hepatectomized adult rats (Craddock, 1973) or to rats previously treated with a single dose of carbon tetrachloride (Pound, et al. 1973) induced liver tumors. Both treatments induce liver cells to divide and these observations prompted Craddock (1973) to speculate that both injury to the genetic material and the occurrence of cell replication before the damage has been repaired are required for carcinogenesis. However, the incidence of liver tumors following chronic administration of either nitrosodimethylamine or nitrosodiethylamine was the same in both intact and partially hepatectomized rats (Rajewski, et al. 1966). There seems to be no simple explanation as to why a single oral dose of nitrosodimethylamine, while ineffective in the adult mouse or rat, is capable of inducing liver tumors in the adult Syrian golden hamster (Tomatis and Cafis, 1967).

Some N-nitroso compounds administered during pregnancy induce cancer not only in the mother but also in the offspring. A single administration of N-nitrosoethylurea to pregnant rats resulted in malignant tumors of the vagina, uterus or ovaries. Given on days 15 through 18 of gestation (but not before day 11) the compound produced brain and spinal cord tumors in the offspring. Ethylurea and nitrite given orally to pregnant rats also

produced nervous system tumors. The sensitivity of the nervous system during prenatal development was estimated to be about 50 times that of adults (Druckrey, et al. 1969). Exposure during days 10 through 21 of gestation led to renal tumors in the offspring several months after treatment (Shank, 1975). The N-nitrosoamines, including nitrosodimethylamine, nitrosodiethylamine, nitrosomethylbutylamine, nitrosoethylvinylamine and nitrosopiperidine have induced tumors in the offspring of mice, rats and Syrian golden hamsters only when administered during the last days of pregnancy. Subcutaneous, intraperitoneal, intravenous, and oral administration and inhalation were equally effective (Tomatis, 1973). In rats the tumors observed were mainly neurogenic. However, Mohr, et al.(1966) observed tracheal papillomas in almost half the offspring of pregnant Syrian golden hamsters within 25 weeks of subcutaneous administration of N-nitrosodiethylamine on days 9 through 15 of gestation. In mice, treatment with nitrosodiethylamine on day 16, 17 or 18 of gestation induced mainly lung tumors. It has been suggested that the inefficacy of the nitrosamines in early pregnancy is due to the lack in the fetus of enzyme systems necessary for metabolic activation (Druckrey, 1973b). Presumably, although active products are produced in the maternal tissues, they are generally too unstable to survive crossing the placenta and hence do not affect the fetus.

Exposure to N-nitrosamides during pregnancy may result in a risk not only to the immediate offspring but for at least two more generations of animals. An increased incidence of tumors has been reported in the F_1, F_2 and F_3 descendants of rats treated with N-nitrosomethylurethane or

N-nitrosomethylurea during pregnancy (Montesano and Bartsch, 1976). There is no experimental evidence to indicate that N-nitrosamines pose a similar threat.

Nitrosodiethylamine has been found in the stomach contents of suckling rats following oral administration to the dam. The young rats subsequently developed multiple tumors (Schoental and Appleby, 1973).

The carcinogenic action of the N-nitroso compounds can be modified by appropriate treatment. The effect of partial hepatectomy or prior administration of carbon tetrachloride has already been mentioned. Other interactions have also been demonstrated. The intragastric administration of methylcholanthrene to mice (which would be expected to increase the activity of liver nitrosamine-metabolizing enzymes) together with intraperitoneal injection of nitrosodimethylamine resulted in increased incidence and decreased latency period to tumors as compared with mice treated with either compound alone (Cardesa, et al. 1973). Intratracheal instillation of ferric oxide and subcutaneous injection of nitrosodimethylamine in Syrian golden hamsters induced esthesioneuroepitheliomas of the nasal cavity, a type of tumor not induced in hamsters by nitrosodimethylamine alone (Stenbäck, et al. 1973). Ferric oxide is frequently used as a carrier for introducing carcinogenic chemicals into the lung by intratracheal instillation. It is believed to facilitate the penetration and retention of the carcinogen in the lung tissue. However, in the present instance, ferric oxide can be considered a cocarcinogen. Other studies have shown enhanced bronchial metaplasia and tracheal papilloma formation

in hamsters treated with nitrosodiethylamine by subsequent exposure to cigarette smoke, volatile acids, aldehydes, and methyl nitrite and increased incidence of lung tumors by subsequent intratracheal instillation of benzo(a)pyrene and/or ferric oxide particles (Magee, et al. 1976). The toxicity and carcinogenicity of various alkylnitrosoureas are said to be increased when administered with copper, nickel or cobalt ions (Magee, et al. 1976). Magee, et al. (1976) cite examples of agents known to depress the activity of drug metabolizing enzymes which have been reported to modify the action of N-nitrosamines. A protein-deficient diet protected against acute liver damage in rats and resulted in an almost two-fold increase in the LD_{50}, however the incidence of kidney tumors in survivors was 100 percent. Aminoacetonitrile, which inhibits the metabolism of nitrosodimethylamine both *in vivo* and *in vitro*, prevented its toxic and carcinogenic effect in rat liver. At the present time, these interactions appear to be of academic rather than practical interest.

Although there is a wealth of reported studies on the carcinogenicity of N-nitroso compounds, these tend to address structure-activity relationships or mechanisms of action: information on dose-response characteristics is sparse. Table 4 includes experimental data culled from studies in the published literature in which nitrosamines were administered over the lifetime of experimental animals at two or more daily dose levels which induced tumors in some but not all animals exposed. Although only tumors (benign and malignant) occurring in the principal target organ are listed, in all cases other organs were also affected. Some comments are necessary. The data of Druckrey, et al. (1963) are difficult to interpret since many

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Table 4. - Dose-response data from studies involving lifetime exposure to four N-nitrosamines

	Daily Dose (mg/kg Body wt.)	Animals with tumors (Animals exposed) Male	Female
Compound: N-Nitrosodimethylamine			
Vehicle: Diet	0	0(12)	0(29)
Species: Rat	0.067	1(19)	
Target organ: Liver	0.12		0(18)
(Terracini, et al. 1967)	0.17	1(6)	
	0.30		4(62)
	0.60		2(5)
	1.2		15(23)
	6.0		10(12)
Compound: N-Nitrosodiethylamine			
Vehicle: Drinking water	0.075	5(60)	
Species: Rat	0.15	22(45)	
Target organ: Liver	0.30	63(80)	
(Druckrey, et al. 1963)	0.60	51(60)	
	1.2	36(40)	
Compound: N-Nitrosodi-n-butylamine			
Vehicle: Drinking Water	7.6	17(47)	
Species: Mouse	8.2		2(42)
Target organ: Bladder	29.1	36(45)	
(Bertram and Craig, 1970)	30.9		8(45)
Compound: N-Nitrosopyrrolidine			
Vehicle: Drinking Water	0	0(61)	
Species: Rat	0.30	3(60)	
Target organ: Liver	1.0	17(62)	
(Preussmann, et al. 1977)	3.0	31(38)	

animals were lost through intercurrent infections. Thus, of the 60 animals orginally exposed to nitrosodiethylamine at the 0.075 mg/kg body weight level, 40 succumbed to a "pneumonia-infection" during the first 600 days of the experiment and, by the time the first (and only) hepatic carcinoma had been identified in this group, there were only three survivors. The sex of the animals used in this study is not specified. However, it is probable that they were male BD II (albino) rats. In addition to tumors of the urinary bladder, Bertram and Craig (1970) report a very high incidence of esophageal tumors unrelated to dose following administration of N-nitrosodi-n-butylamine. The incidence of bladder tumors in females was relatively low but these tumors developed significantly later than in males. The authors speculate that, had not death from esophageal tumors intervened, both sexes would have had a uniformly high bladder tumor incidence. Preussman, et al. (1977) report that other dose response studies (initially with N-nitrosopiperidine) are under way or planned. In other studies with nitrosodimethylamine, mink, apparently the most sensitive species, developed tumors when fed 0.05 mg/kg body weight 2 days per week (Natl. Acad. Sci., 1978). An increase in the incidence of malignant liver and kidney tumors was found in male but not female rats, and not in mice of either sex, when the animals continuously inhaled air containing 200 $\mu g/m^3$ of dinitrosomethylamine for 17 months (mice) or 25 months (rats). A concentration of 5 $\mu g/m^3$ produced no increase in tumors (Natl. Acad. Sci., 1978). Preussman, et al. (1977) have attempted to derive "no-effect-levels" for rats for nitrosodimethylamine and other carcinogenic nitrosamines (although they themselves question the validity of such levels). Expressed as dietary levels, the estimates are: nitrosodimethylamine, 1 to 2 mg/kg;

nitrosodiethylamine, < 1 mg/kg; N-nitrosopyrrolidine, 3 to 5 mg/kg (corresponding to a daily intake of approximately 0.1, < 0.1, and 0.3 mg/kg body weight, respectively).

Attempts have been made to derive some measure of the relative carcinogenic potency of N-nitroso compounds in the absence of complete dose-response information. The favored data base is the review of Druckrey, et al. (1967) of studies in which adult BD rats received small daily doses (usually orally) of 51 N-nitrosamines and 13 N-nitrosamides. Druckrey, et al. calculated the mean total carcinogenic dose required for production of tumors in 50 percent of the animals (D_{50}). Wishnok and Archer (1976) have used only those D_{50} values corresponding to a daily dose that was approximately a constant fraction (1 to 3 percent) of the acute oral LD_{50} (a dose which gave a mean induction time for appearance of tumors of about 300 to 600 days), and, in order to have increasing carcinogenicity represented by increasing (and manageably small) numbers, have expressed carcinogenic potency as log ($1/D_{50}$). Table 6 lists the values given by Wishnok and Archer (1976) for most of the carcinogenic N-nitroso compounds examined by Druckrey, et al. (1967). For the four N-nitrosamines for which dose-response are available the order of increasing potency as measured by log ($1/D_{50}$) is: N-nitrosopyrrolidine (1.41); N-nitroso-n-butylamine (1.61) N-nitrosodimethylamine (2.27); N-nitrosodiethylamine (3.20). Analysis of the experimental dose-response data places these compounds in the same order (Table 5). Despite this (possibly fortuitous) agreement, log ($1/D_{50}$) values can be regarded only as providing general guidance.

Wishnok and Archer (1976) and Wishnok, et al. (1978), using the log

($1/D_{50}$) value as a measure of carcinogenicity have attempted to relate carcinogenicity to the chemical and physical properties of N-nitroso compounds. Wishnok, et al. (1978) have derived an equation which takes into account, not only chemical structure but also the partition coefficient of the N-nitroso compounds, and their electronic factors as expressed by Taft σ^* values of substituents on the α-carbon atoms. With certain explainable exceptions, which are discussed by Wishnok, et al., the equation appears to serve as a reasonably reliable method for assessing carcinogenicity.

Human Data: There is no instance known of occupational exposure to specific nitrosamines having resulted in a cancer in man. The epidemiologic evidence for the association of N-nitroso compounds with human cancer is also very limited. These data have been reviewed by a panel convened by the National Academy of Sciences (1978) and the following is taken in its entirety from this report.

> A few epidemiological studies have attempted to associate environmental nitrates, nitrites, and nitroso compounds with human cancer. A problem common to all the early studies was the inability to measure with high specificity N-nitroso compounds in biological samples. For example, African studies associating esophageal cancer with a nitrosamine in a local alcoholic beverage (McGlashan, 1969) and a study relating carcinoma of the cervix with nitrosamine formation in the vagina of South African women (Harington, et al. 1973) were done without the advantage of mass spectroscopic confirmation that is needed to identify the nitrosamines.
>
> The International Agency for Research on Cancer has investigated the possible association between N-nitroso compounds in the diet and esophageal cancer in specific areas of Iran and France, where these tumors occur at a high rate, and in nearby areas where the tumor rates are not elevated (Bogovski 1974). Complete studies of possible sources of exposure to the carcinogens have

not been made, but 15 of 29 samples of cider contained 1 to 10 μg/kg DMN (nitrosodimethylamine) and two samples also contained DEN (nitrosodiethylamine) (less than 1 μg/kg). Correlations between dietary intake of N-nitroso compounds and incidence of esophageal cancer have not yet been made.

The Chinese conducted a similar study in the Anyang region, where it is claimed that approximately 20 percent of all deaths (not just cancer deaths) result from esophageal cancer (Coordination Group, 1975). Twenty-three percent of the food samples from areas with the highest cancer rates were reported to contain DMN, DEN, and methylbenzyl nitrosamine. However, confirmation of this analysis by gas chromatography and mass spectroscopy is required before the finding can be accepted. Dietary nitrite levels were higher in areas of high cancer incidence than in low incidence areas. Chickens in areas where there were high rates of esophageal cancer in humans also had a high incidence of similar tumors, suggesting an environmental etiology for the disease.

Zaldivar and Wetterstand (1975) demonstrated a linear regression between death rates from stomach cancer and the use of $NaNO_3$ as fertilizer in various Chilean provinces. Fertilizer use was presumed equitable to human exposure to nitrates and nitrosamines, but no actual exposure data were reported. Armijo and Coulson (1975) have shown similar correlations. These reports suggest that nitrate from fertilizer enters the diet in meat, vegetables, and drinking water, is reduced to nitrite by microbial action, and thus is available for in vivo nitrosation of secondary amines in the diet, to form carcinogenic nitrosamines, which induce stomach cancer. As yet, no scientific data have been gathered that support this hypothesized etiology, and the suggested causal relationship remains highly speculative.

Hill, et al. (1973) correlated differences in rates of stomach cancer with the nitrate content of drinking water in two English towns; but again, the evidence required to demonstrate a causative role for nitrate is not available. Gelperin, et al. (1976) compared death rates ascribed to cancer of the gastrointestinal tract and liver with nitrate levels of drinking water in three unmatched population groups in Illinois used in an infant mortality study. No significant differences in cancer rates were found among the three groups (the level of significance was not stated). It is doubtful, however, whether the available mortality data permitted an analysis that could have detected an effect in the high nitrate population.

Increased rates of stomach cancer have been observed in Japan in occupational groups and other populations characterized by an unusually high consumption of salt-preserved foods (Sato, et al. 1959); presumably, these foods are high in nitrate and perhaps in nitrite.

A statistical correlation is presented of the incidence of cancer mortality with estimated exposures of urban populations in the United States to various environmental and dietary factors. Again to quote the report (Natl. Acad. Science, 1978): "Strong positive correlations were shown between the aggregate rate of cancer mortality and components of the diet, particularly nitrite and protein; however, insufficient biological evidence is available to confirm the hypothesized causal pathway (involving formation of N-nitroso compounds from nitrite and amines, reacting in the stomach)." There is, in fact, direct evidence for formation of nitrosamines from precursors in the human stomach (see, for example, Fine, et al. 1977b): still in contention is the extent to which nitrosation occurs.

Mechanism of action: Although N-nitrosamines such as N-nitrosodimethylamine and N-nitrosomorpholine are rapidly and fairly evenly distributed throughout the bodies of rats after injection (Magee, 1972; Stewart, et al. 1974), the acute toxic damage they produce is more severe in the liver than elsewhere, and tumors following chronic exposure are confined mainly to the liver and kidney (Druckrey, et al. 1967). In vitro studies have shown that the liver and kidney possess the greatest capacity for metabolism of N-nitrosodimethylamine (Montesano and Magee, 1974). These observations are most readily explained on the assumption that carcinogenesis and other biological actions of nitrosamines are mediated by metabolic products. The lack of mutagenic activity exhibited by nitrosamines in bacterial test systems in the absence of a metabolic activating system (Montesano and Bartsch, 1976) supports this hypothesis, which is now generally accepted.

The N-nitrosamides differ from the N-nitrosamines in that they are chemically unstable at physiological pH, and decompose non-enzymically, again into active metabolic products, upon contact with the tissues. They therefore tend to produce damage or tumors at the site of administration.

The nature of the metabolite(s) responsible for the carcinogenic activity of N-nitroso compounds is still in debate. Magee (1977) has adduced considerable evidence in support of the commonly accepted hypothesis that the active agents are electrophilic alkylating agents which bind to DNA. The major product formed in rat liver after administration of nitrosodimethylamine or nitrosodiethylamine is the corresponding 7-alkylguanine. However, little correlation has been found between the occurrence of 7-alkylguanine in DNA and the tumor-producing activity of the nitrosamines. A much better correlation has been demonstrated between the formation and persistence of O^6-alkylguanines and tumor incidence (Pegg and Nicoll, 1976). These authors postulate that the formation and persistence until cell division of certain promutagenic products such as O^6-methylguanine might be responsible for the initiation of tumors and that the differing abilities of various tissues to catalyze DNA repair might account for part of the differing susceptibilities of these tissues to the carcinogenic action of the N-nitroso compounds (Pegg and Nicoll, 1976). However Lijinsky and coworkers found that a series of cyclic nitrosamines, while equally carcinogenic with the aliphatic nitrosamines, gave rise to much less alkylated guanines: in some cases none could be detected (Lijinsky, 1977). For this and other reasons, Lijinsky concludes that the initial step cannot be a simple alkylation of DNA.

Summary. Many N-nitrosamines (and N-nitrosamides) are teratogenic, mutagenic, and/or carcinogenic. Evidence from experimental animals suggests that, as carcinogens, they are most effective by the oral route and when given as multiple small doses. However, some are capable of inducing tumors after a single dose and they are also capable of inducing tumors in certain organs and tissues regardless of the route of administration, i.e., they are systemic carcinogens. In the rat, at least, every organ is probably susceptible to tumor induction by some nitrosamine. There is a strong relation between chemical structure and type of tumor induced. There are large differences in tumor response among species, both in type of tumor produced and in susceptibility.

The late fetus and neonate appear to be highly susceptible to the carcinogenic action of both N-nitrosamines and N-nitrosamides. The sensitivity of the nervous system to some N-nitrosamides during prenatal development is about 50 times that in the adult. A single exposure to some nitrosamides during pregnancy may result in development of tumors not only in the immediate descendants but in at least two succeeding generations. Although prolonged exposure to some nitrosamines is needed to elicit tumors in adult animals, a single dose of the compound will induce tumors in the newborn.

The epidemiological studies to date have been inadequate to establish any correlation between exposure to N-nitroso compounds or their precursors and human cancer as valid causal relationships. Nevertheless, the ability of N-nitrosamines to induce tumors in a wide range of species other than

man, together with the fact that human liver tissue is capable of forming alkylating and mutagenic metabolites, suggest strongly that it is improbable that humans are refractory to the carcinogenic action of these compounds.

CRITERION FORMULATION

Existing Guidelines and Standards

Current Levels of Exposure

For the general population exposure information is very limited. It has been estimated that air, diet and smoking all play a roughly equivalent role in direct human exposure, contributing a few micrograms per day, with direct intake from ingested water probably much less than 1 μg per day (U.S. EPA, 1976).

There is even greater uncertainty with regard to the significance of exposure to precursors. The chief source of the nitrate body burden, except in the newborn, is ingested vegetables, unless rural well water high in nitrate is consumed. Food and water normally contribute a few hundered milligrams per day. Inhalation may also contribute several hunder micrograms per day (U.S. EPA, 1977). On a daily basis, the major source of nitrite is saliva. However, salivary nitrite is presented to the body as a continuous, low-level input, in comparison with the relatively high concentrations over short periods resulting from ingestion of cured meats. This may be significant since the rate of nitrosation is a function of the square of the nitrite concentration (U.S. EPA, 1977).

The concentrations of nitrite (and its precursors, ammonia and nitrate) and nitrosatable compounds can be much greater in soils heavily fertilized with organic waste matter

or in waters receiving runoff from agricultural areas or discharges of industrial or municipal waste waters containing substantial amounts of amines. Levels of nitrate or municipal drinking water in the U.S. seldom exceed 10 mg/l nitrate N, although some private supplies contain much more.

Significant concentrations of nitrosamines have been reported for a limited number of samples of ocean water, river water and waste treatment plant effluent adjacent to or receiving wastewater from industries using nitrosamines or secondary amines in production operations. Nitrosodimethylamine has been reported at the 3-4 ng/l level in these samples. Nitrosamines, however, are rapidly decomposed by photolysis and do not persist for a significant time in water exposed to sunlight.

Although it is difficult to analyze this wide spectrum of exposure potential, it must be concluded that ingested water is a relatively minor source of exposure when compared with other potential sources of either preformed N-nitroso compounds or their precursors.

Special Groups at Risk

Because of the ubiquitous nature of nitrosatable compounds and nitrosating agents in the environment (food, air, drugs, tobacco, water, soil) special risk groups would have to include those individuals who are exposed to multiple exposure situations. To quantify this, however, is almost impossible at this point because of the need to create exposure scenarios for which the bounding factors are unknown or relatively wide ranging.

Basis and Derivation of Criterion

Both N-nitrosamines and N-nitrosamides exhibit acute toxicity, teratogenicity, mutagenicity and/or carcinogenicity. For most, it is the latter capability which demands consideration in the context of human exposure since the toxicological evidence is such that they must be treated as potential human carcinogens. Thus, nitrosamines are included in the American Conference of Governmental Industrial Hygienists' (1977) List of "Industrial Substances Suspect of Carcinogenic Potential for Man." No Threshold Limit Value is given. The guidelines which follow are based upon the assumption that N-nitrosamines are human carcinogens.

Adequate dose-response data to permit an assessment of the carcinogenic risk to man are available from studies involving lifetime exposure of rats or mice to four nitrosamines (N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosodi-n-butylamine, and N-nitrosopyrrolidine) in their drinking water or food (see Table 4 of the document). These data have been used to derive estimates of the concentrations in water which, if used as the source for man of drinking water and edible fish and shellfish, would increase the risk of a tumor by not more than one in 100,000 individuals exposed for the duration of their lifespan. The method of extrapolation and the mathematical model (a linear, non-threshold model) used are described in the Appendix. The water criteria numbers given in Table 5 are based upon the tumor incidence, at the organ which is the chemical's principal site of action (see Table 4 of the document), in the

most sensitive sex, at the lowest dose level which yielded a response significantly greater than in the control animals. The actual parameters used are given in Appendix I.

The only nitrosamine for which any data on bioaccumulation in fish or shellfish are available is N-nitrosodiphenylamine. One test in bluegill yielded a maximum bioconcentration factor (BCF) of 217 for the whole fish (U.S. EPA, 1978). Using this, a weighted BCF for all species was calculated to be 500. These values are believed not to be representative for the nitrosamines for which carcinogenicity data are available and another approach has therefore been attempted. A relationship has been described between the BCF and the n-octanol/water partition coefficient, Log BCF = 0.76 Log P - 0.23, (Veith, et al. Manuscript). The equation can be used to estimate the BCF for aquatic organisms that contain about 8 percent lipids from the partition coefficient P. The partition coefficients for N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosodi-n-butylamine and N-nitrosopyrrolidine are 0.27, 3, 83, and 0.65 respectively (Singer, et al. 1977a). Values of BCF calculated using this equation are 0.2, 1.4, 17, and 0.42 respectively, for N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosodi-n-butylamine, and N-nitrosopyrrolidine respectively. An adjustment factor of

2.3/8.0 = 0.2875 can be used to adjust the estimated BCF from the 8.0 percent lipids on which the equation is based to the 2.3 percent lipids that is the weighted average for consumed fish and shellfish.

The adjusted BCF values are 0.06, 0.39, 4.9, and 0.12 respectively.

Other empirical procedures (Neely, et al. 1974) can be used to estimate BCF's from partition coefficients. The values obtained are of the same order of magnitude.

For purposes of calculating criteria, the estimated BCF's for the methyl, ethyl, butyl, and pyrrolidine compounds are judged to be rough estimates of the bioconcentration potential. As the influence of these values ranges from 5/100 percent to 4 percent of the exposure uptake from fish consumption, for simplicity the assumed BCF for criteria level purposes is zero.

TABLE 5

Concentrations in water estimated to induce no more than one excess cancer per 100,000 individuals exposed over a lifetime

Compound	Estimated Concentration (µg/l)	Data base
N-Nitrosodimethylamine	0.026	Rats (female) (Druckery, et al. 1967)
N-Nitrosodiethylamine	0.0092	Rats (male?) (Druckrey, et al. 1963)
N-Nitrosodi-n-butylamine	0.013	Mice (male) (Bertram and Craig, 1970)
N-Nitrosopyrrolidine	0.11	Rats (mixed sexes) (Preussman, et al. 1977)

Table 3 of the document lists one measure of the relative carcinogenic potential of a number of N-nitrosamines. The value of N-nitrosodiethylamine (3.20) is exceeded by only that for N-nitrosomethyl-2-chloroethylamine (3.21) and approached by only the values for N-nitroso-methylbenzylamine (3.10) and N-nitrosomethyl-(2,phenylethyl)amine (3.01). Hence, N-nitrosodiethylamine can reasonably be considered to be one of the most carcinogenic nitrosamines. It is therefore appropriate to base the water criterion number for any individual nitrosamine on the number obtained for N-nitrosodiethylamine, viz. 9.2 ng/l.

This criterion number has been derived by considering only the excess cancer risk imposed by exposure to contaminated drinking water, fish, and shellfish. However, the average daily intake of preformed nitrosamines from other sources (air, diet, and smoking) is estimated to be in the order of a few micrograms per day (U.S. EPA, 1976). There is an additional and, at the present time, ill-defined contribution to the body burden from the in vivo nitrosation of precursors. This contribution has been variously estimated to range from a few micrograms to several hundred micrograms daily (Natl. Acad. Sci., 1978). Thus, present evidence suggests that control of exposure to N-nitrosamines should take into account both preformed nitrosamines and their precursors in the environment.

Under the Consent Decree in NRDC v.Train, criteria are to state "recommended maximum permissible concentrations (including where appropriate, zero) consistent with the

protection of aquatic organisms, human health, and recreational activities." Nitrosamines are suspected of being human carcinogens. Because there is no recognized safe concentration for a human carcinogen, the recommended concentration of nitrosamines in water for maximum protection of human health is zero.

Because attaining a zero concentration level may be infeasible in some cases and in order to assist the Agency and States in the possible future development of water quality regulations, the concentrations of nitrosamines corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides an estimate of the additional incidence of cancer that may be expected in an exposed population. A risk of 10^{-5} for example, indicates a probability of one additional case of cancer for every 100,000 people exposed, a risk of 10^{-6} indicates one additional case of cancer for every million people exposed, and so forth.

In the Federal Register notice of availability of draft ambient water quality criteria, EPA stated that it is considering setting criteria at an interim target risk level of 10^{-5}, 10^{-6} or 10^{-7} as shown in the table below.

Exposure Assumptions (per day)	Risk Levels and Corresponding Criteria (1)			
	0	10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 18.7 grams fish and shellfish. (2)				
N-nitrosodimethylamine	0	.00026 µg/l	.0026 µg/l	.026 µg/l
N-nitrosodiethylamine	0	.000092 µg/l	.00092 µg/l	.0092 µg/l
N-nitrosodi-n-butylamine	0	.00013 µg/l	.0013 µg/l	.013 µg/l
N-nitrosopyrrolidine	0	.0011 µg/l	.011 µg/l	.11 µg/l
Consumption of fish and shellfish only.				
N-nitrosodimethylamine	0			
N-nitrosodiethylamine	0			
N-nitrosodi-n-butylamine	0			
N-nitrosopyrrolidine	0			

(1)Calculated by applying a modified "one-hit" extrapolation model described in the Methodology Document to the animal bioassay data presented in Appendix I and in Table 10. Since the extrapolation model is linear at low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concentrations corresponding to other risk levels can be derived by multiplying or dividing one of the risk levels and corresponding water concentrations shown in the table by factors such as 10, 100, 1,000, and so forth.

(2) Approximately zero percent of Nitrosamine exposure results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of zero.

The remaining 100 percent of Nitrosamine exposure results from drinking water.

Concentration levels were derived assuming a lifetime exposure to various amounts of nitrosamines, (1) occurring from the consumption of both drinking water and aquatic life grown in waters containing the corresponding nitrosamines concentrations and, (2) occurring solely from consumption of aquatic life grown in the waters containing the corresponding nitrosamines concentrations. Because data indicating other sources of nitrosamines exposure and their contributions to total body burden are inadequate for quantitative use, the figures reflect in incremental risks associated with the indicated routes only.

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APPENDIX II

Derivation of Criterion for Dimethylnitrosamine

Druckery (1967) summarized a series of experiments in which a large series of nitrosamine compounds were given to B-D rats for a lifetime. He found that the incidence of liver tumors increased with daily dose, d, and that the median time when tumors were observed, t_{50}, was less at higher doses and the relationship between d and t_{50} was $d(t_{50})2.3 = k$, where k is a constant equal to 0.81×10^4 mM/kg/day when t_{50} is expressed in units of days. The extrapolation model uses the dose units of mg/kg/day and the time units of fractions of a lifetime. Converting k to these units by using 728 days as the lifetime and a molecular weight of 74 mg/mM gives the following:

$$k = \frac{0.81 \times 10^4 \text{ mM/kg/day} \times 74 \text{ mg/mM}}{(728)\ 2.2} = 0.30271$$

Therefore the parameters of the dose-response model are:

$n_t/N_t = 0.5$ $\qquad$ $L = 728$ days

$n_c/N_c = 0$ $\qquad$ $_{dt}n = 0.30271$

$w = 0.35$ kg $\qquad$ $R = 0$

The result is that the water concentration should be less than 0.026 micrograms per liter in order to keep the individual lifetime risk below 10^{-5}.

Derivation of Criterion of Diethylnitrosamine

Druckery, et al. (1963) administered diethylnitrosamine to B-D rats via drinking water in nine dose groups ranging from .075 to 14.2 mg/kg/day.

They found that the incidence of liver tumors increased with daily dose, d, and that the median time when tumors were observed, t_{50}, was less at higher doses and the relationship between d and t_{50} was $d(t_{50})^{2.3} = k$, where k is a constant. At a dose of 0.6 mg/kg/day the incidence of tumors reached 50 percent (30 animals with liver tumors out of 60 animals initially) at 355 days. Using a bioaccumulation factor of zero the parameters of the extrapolation model are:

$n_t = 30$	d = 0.6 mg/kg/day
$N_t = 60$	w = 0.28 kg
$nc/N_c = 0$	L = 730 days
Le = 355 days	m = 2.3 instead of 3 in the standard model
le = 355 days	$dt^m_{R=0} = 0.6\ (355/730)^{2.3} = 0.114$

The result is that the water concentration should be less than 0.0092 micrograms per liter in order to keep the individual lifetime risk below 10^{-5}.

Derivation of Criterion for Dibutylnitrosamine

Bertram and Crain (1970) administered dibutylnitrosamine via drinking water to C57BL/6 mice at dose levels of about 8 and 30 mg/kg/day until the animals became moribund or died. They found that all of the 179 animals reaching autopsy had tumors of the bladder of espohagus except three, which had tumor induction at either site was the males given 7.6 mg/kg/day, where the mean tumor induction time was 261 days. Assuming a bioaccumulation factor of zero, the parameters of the extrapolation model are:

$n_t/N_t = 0.5$	d = 7.6 mg/kg/day
$n_c/N_c = 0$	L = 728 days
Le = 261 days	w = .028 kg
le = 261 days	R = 0

The result is that the water concentration should be less than 0.013 micrograms per liter in order to keep the individual lifetime risk below 10^{-5}.

5800
499

Derivation of Criterion of N-Nitroso-pyrrolidine

Preussman, et al. (1977) found a dose-related incidence of hepatocellular carcinomas in Sprague-Dawley rats in a lifetime feeding study at levels of 0.3, 1.0, 3.0, and 10 mg/kg/day. The total tumor incidence was equal to controls at the 0.3 mg/kg/day level, but was 46, 84 and 32 percent at the successively higher doses. Animals at the highest dose had a smaller tumor incidence because of early deaths from pneumonia. At 3 mg/kg/day the incidence of liver tumors was 31/38 whereas the 61 controls had no tumors. With a negligible fish accumulation, the parameters of the dose-response model are:

nt = 31	le = 104 weeks
N_t = 38	d = 3 mg/kg/day
n_c = 0	w = 0.35 kg
N_c = 61	L = 104 weeks
Le = 104 weeks	R = 0

The result is that the water concentration should be less than 0.11 micrograms per liter in order to keep the individual lifetime risk below 10^{-5}.